DOCKET NO. 9958-002-27 CONT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: JOHN N. SHANNON, ET AL. ART UNIT: 1632
SERIAL NO.: 09/811,509 EXAMINER: CHIN, SHIN LIN
FILING DATE: MARCH 20, 2001
FOR: METHODS AND ORGANISMS FOR CONCENTRATING AND RECOVERING METALS AND MINERALS FROM AQUEOUS MEDIA

DECLARATION UNDER 37 C.F.R. § 1.132

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

I, Martin Alan Winkler, Ph.D., do hereby declare and state that:

1. I am currently a Principal Scientist at Abbott Laboratories in Abbott Park, Illinois. I have substantial experience in the supervision and conduct of research directed to the identification, purification, and characterization of purified natural-source compounds. I have developed bioanalytical methods for characterizing proteins and other molecules. I have published extensively in the field regarding the isolation and characterization of a variety of molecules. I am a co-inventor of four patented inventions relating to the identification, isolation, purification, and characterization of different molecules. My curriculum vitae is attached.

2. I am not a co-inventor nor do I have any ownership interest in the present patent application of John N. Shannon, et al. I have reviewed the subject matter contained in the present application.

3. I understand that Claims 23, 26, 28, 29, and 31 are currently rejected under 35 U.S.C.

§112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it pertains, or with which it is most nearly connected, to make and/or use the invention. I have carefully reviewed each of the rejected claims in light of the disclosure of the invention provided by the specification.

4. Based on my extensive experience and education, I DECLARE that it is reasonable to conclude as follows.

AT THE TIME OF THE INVENTION, ONE OF ORDINARY SKILL IN THE ART WOULD HAVE BEEN ABLE TO PRACTICE THE FULL SCOPE OF THE CLAIMED INVENTION WITHOUT UNDUE EXPERIMENTATION FOLLOWING THE GUIDANCE PROVIDED IN THE SPECIFICATION OF THE FILED PATENT APPLICATION.

5. The present application specification expressly demonstrates and incorporates by reference the disclosure that sponges are well known to harbor bacteria that are of the same genus as bacteria, which are capable of concentrating metals or minerals. The present specification also provides ample support for the methods necessary to promote the growth and cultivation of sponges, which are known to have symbiotic relationships with bacteria. The methodology necessary to cultivate sponges that are capable of maintaining a symbiotic relationship with bacteria, some of which are of the same genus as known metal or mineral concentrating bacteria, is well-demonstrated and easily repeatable by one of ordinary skill in the art with minimal experimentation. The specification of the present application clearly teaches that genes that encode proteins, which effect bio-accumulation of metals and minerals have been identified, isolated, and inserted in organisms. The successful modification of these transgenic organisms to be active bio-

accumulators of the target metals is also known to one of ordinary skill in the art. The specification also teaches that the cell wall precious metal binding sites have been identified for some bacteria as teichoic and teichuronic acids. A listing of numerous bacteria species and the metals that they are capable of concentrating is also provided in the specification. The specification correctly teaches that the isolation and identification of genes responsible for accumulating the metals in each of the identified bacteria species can be done using well-known molecular cloning techniques. Further, the specification teaching that the preparation of transgenic microorganisms, which incorporate the isolated gene of choice, can also be accomplished using well-known techniques is a reasonable assertion given the past success cited in the specification and known to one of ordinary skill in the art. Page 3, line 26 through page 6, line 14. provides sufficient guidance and evidence of success in representative steps of gene isolation and identification, transgenic organism preparation, and cultivation of sponges having a symbiotic relationship with bacteria of the same genus (Cyanobacter) as known metal bio-accumulators. Provided with such guidance, one of ordinary skill in the art would be able to practice the claimed invention using techniques and process well-known in the art with a high degree of confidence in the successful outcome of the endeavor. Importantly, I do not know of any reason disclosed in the specification or in any reference of record that would cast doubt on the truth of the teachings of the present application. In view of the above, I believe that it would not be burdensome to follow the guidance provided in the present application to obtain the results of the claimed invention.

6. In declaring no undue experimentation, I have considered the legal standard for determining a finding of undue experimentation as set forth in In re Wands, 858 F.2d 731, 8

USPQ2d 1400 (Fed. Cir. 1988). I have given full consideration to the factors enunciated in that decision in determining that the specification in the filed patent application is enabling for the full scope of the originally filed claims. I have reviewed the facts associated with the Wands decision, including the claims and specification of United States Patent No. 4,879,219 (the '219 patent). A copy of that legal decision and the '219 patent are attached.

7. According to the '219 patent, monoclonal antibodies are formed from hybridomas and the starting materials included mice, myeloma cells, antigen, etc. are then carried through a cell fusion process followed by two levels of screening to identify whether an antibody is produced falling within the scope of the claim. At the time of the invention in the '219 patent, the amount of experimentation required to find the antibodies falling within the scope of the claim in that patent was substantial.

8. By way of contrast, according to the methodology in the specification of the filed patent application, any experimentation required would be much simpler. The methodology according to the '219 patent requires far more work including immunizing mice, removal of their spleen, separation of the lymphocytes from other spleen cells and then formation of fusion cells of the lymphocytes with myeloma cells to form hybridomas. In the '219 patent, this is all preliminary, prior to any screening. It is my considered belief that the quantity of experimentation to find successful candidate antibodies according to the '219 patent at the time of that invention is substantially more than would be needed to employ the disclosure of the invention provided in the present application to obtain the claimed invention.

All statements made herein of my own knowledge are true and all statements made on information and belief are believed true. Furthermore, I am aware that willful false statements and

the like are punishable by fine, imprisonment or both, 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the above-captioned patent application, and any patent to issue thereon.

Date: 4/26/2003

Martin Alan Winkler, Ph.D.

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EDUCATION

B.A. The University of Texas at Austin, Austin, TX Major: Biology.

Ph.D. The University of Texas at Austin, Austin, TX Immunology-Immunochemistry
Purification and immunochemical characterization of beta-2-microglobulin from chicken sera

CAREER SUMMARY

Identification, purification, and characterization of purified natural-source and recombinant proteins for immunoassay and for therapeutics. Development of bioanalytical methods for characterizing proteins and other molecules for FDA submissions. Immunoassay development. Production, purification, and analysis of monoclonal antibody heteroconjugates. Analysis of proteins and identification of microorganisms by mass spectrometry.

PROFESSIONAL EXPERIENCE

Principal Scientist
May 1999 to present-Abbott Laboratories, Diagnostics Division
ADD Analytical Research—Responsible for macromolecular analysis (proteins, peptides) in Core R& D Analytical research and laboratory.

Senior Research Biochemist
April 1997 to April 1999—Abbott Laboratories, Diagnostics Division
ADD Analytical Research--Protein characterization and methods development for proteins in immunoassays. Analysis by mass spectrometry, capillary electrophoresis, HPLC, and other protein chemistry techniques.

Senior Research Analytical Chemist
Dec. 1995 to April 1997--Abbott Pharmaceutical Products Division
Analytical Research and Development--Methods development for Recombinant Pro-Urokinase and other recombinant proteases and protein drug candidates. Drug analytical methods development. All work done in a cGMP environment.

Senior Research Biochemist
Abbott Diagnostics Division, Dec. 1989 *to Dec.1995*
Lead Biochemist—1989-1993. Antigen identification, purification, and characterization for Abbott Chagas Antibody EIA 510(k) submission. Product launched 7/95.
Developed Chagas Radioimmunoprecipitation assay for Abbott Virus Reference Laboratory.
Developed Chagas confirmatory EIA for R & D use.

Lead Scientist— 1994. Recombinant antigen identification, purification, and characterization for CBER-approvable Chagas' disease blood screens.

Lead Biochemist-1995. Maldi-TOF mass spectrometry and capillary electrophoresis methods for recombinant protein characterization.

Staff Scientist
Biotherapeutics, Inc. (now Response Oncologies, Inc.) July 1988-Sept. 1989 Franklin, TN
Co-director of ATAC project to produce, purify, and test monoclonal antibody heteroconjugates as candidates for human cancer immunotherapy. IND for phase I clinicals accepted by FDA.

Staff Scientist
Biotherapeutics, Inc, Biochemistry Section, Oct. 1987-June 1988 Memphis, TN
Supervised two technicians to produce, analyze, and test monoclonal antibody heteroconjugates

Research Associate
St. Jude Children's Research Hospital, Biochemistry Dept., 1981-1987 Memphis, TN
Structure-function studies of calmodulin-dependent enzymes.
Localization of interaction sites of calmodulin for calmodulin-dependent enzymes.

Postdoctoral Fellow of the American Cancer Society
Rockefeller University, Dept. of Developmental and Molecular Biology, 1979-1981 New York, NY Purified and microsequenced H-2 histocompatability antigens. Identified, purified, and characterized cell adhesion molecules involved in embryonic development.

PROFESSIONAL SOCIETIES

American Society for Mass Spectrometry
American Association of Immunologists
American Association for the Advancement of Science
American Chemical Society
Sigma Xi

ORIGINAL ARTICLES

1. **Winkler, M.A.** and Sanders, B.G. Chemical and immunologic characterization of a beta-2-microglobulin-like protein isolated from chicken sera. Immunochemistry 14:615-619, (1977).
2. **Winkler, M.A.**, Merat, D., Tallant, E.A., Hawkins, S., and Cheung, W.Y. Catalytic site of calmodulin-dependent phosphatase from bovine brain residues in subunit A. Proc. Nat.. Acad. Sci. (USA) 81: 3054-3058 (1984).
3. **Winkler, M.A.**, Zysk, J.R., and Cheung, W.Y. Production and characterization of a monoclonal antibody cross-reactive with calmodulin, calmodulin-dependent phophodiesterase, and protein phosphatase. Methods Enzymol. 139:505-518, (1987).
4. **Winkler, M.A.**, Fried, V.A., Merat,.D.L., and Cheung, W.Y. Differential reactivities of lysines in calmodulin complexed to phosphatase. J. Biol. Chem. 262:15466-15471 (1987).
5. Anthony, F.A., **Winkler, M.A.**, Edwards, H.H., and Cheung, W.Y. A quantitative subcellular localization of calmodulin-dependent phosphatase in chick forebrain J.Neuroscience 8:1245-1253 (1988).
6. Pezzi, L., Merat, D.L, , **Winkler, M.A.**, and Cheung, W.Y. Calmodulin-dependent phosphatase preferentially dephosphorylates a 28 kD protein in human platelets. Int. J. Biochem. 21:791-798 (1989).
7. Bush, D.A. and **Winkler, M.A.** Isoelectric focusing of cross-linked monoclonal antibody heterodimers, homodimers, and derivatized monoclonal antibodies. J.Chromatography (Biomedical Applications) 489:303-311 (1989).
8. Foglesong, D.P., **Winkler, M.A.**, Price, J.O, Reagh, S., Marshall, G., Hixson, K., and West, W.H. Preparation and analysis of bifunctional immunoconjugates containing monoclonal antibodies OKT3 and BABR1. Cancer Immunology and Immunotherapy 30:177-184 (1989).

9. **Winkler, M.A.**, Price, J.O., Foglesong, D.P., and West, W.H. Biodistribution and plasma survival in mice of anti-melanoma monoclonal antibody cross-linked to OKT3. Cancer Immunology and Immunotherapy 31:278-284 (1990).
10. **Winkler, M.A.**, Brashear, R.J., Schur, J.D., Hall, H.J., and Pan, A.A. Detection of antibodies to *Trypanosoma cruzi* among blood donors in the Southwestern and Western United States. II. Evaluation of a supplemental enzyme immunoassay and radioimmunoprecipitation assay for confirmation of seroreactivity. Transfusion 35: 219-225 (1995).
11. Brashear, R.J., **Winkler, M.A.**, Schur, J.D., Lee, H., Burczak, J., Hall, H.J., and Pan, A.A. Detection of antibodies to *Trypanosoma cruzi* among blood donors in the Southwestern United States. I. Evaluation of the sensitivity and specificity of an enzyme immunoassay for detecting antibodies to T. cruzi. Transfusion 35:213-218 (1995).
12. **Winkler, M.A.**, Kundu, S., Robey, T.E., and Robey, W.G. Comparative Peptide Mapping of a Hepatitis C viral recombinant protein by capillary electrophoresis and MALDI-TOF Mass Spectrometry. Journal of Chromatography , 744:177-185 (1996)
13. Kundu, S., Fenters, C., Lopez, M., Calfin, B., **Winkler, M.**, and Robey, W.G. Purity testing of recombinant proteins by capillary electrophoresis. Journal of Capillary Electrophoresis, 3:301-307 (1997).
14. **Winkler, M.A.**, Rivera, D.M., Pan, A.A., and Nowlan, S.F. Homology of *Trypanosoma cruzi* clone 36 repetitive DNA sequence to sequence encoding Human RO/SSA 52KD Autoantigen. Parasite. 5: 94-95 1998)
15. **Winkler, M.A.**, Uher, J., and Cepa, S. Direct Analysis and Identification of Helicobacter and Campylobacter species by MALDI-TOF Mass Spectrometry. Analytical Chemistry 71:3416-3419 (1999).
16. Basombrio, M.A., Segovia, A., Ramos, M.P., Esteban, E., Strumpf, R., Jurgenson, P., **Winkler, M.A.**, Sayre, K., and Ferrer, J.F. Endemic Trypanosoma Cruzi infection in Indian populations of the Gran Chaco territory of South America: performance of diagnostic assays and epidemiological features. Annals of Tropical Medicine and Parasitology 93: 41-48 (1999)
17. **Winkler, M.A.**, Xu, N., Wu, H., and Aboleneen, H. MALDI-TOF MS of Chemically modified Recombinant Hepatitis B Surface Antigens. Analytical Chemistry 4:664A-667A (1999).
18. **Winkler, M.A.**, Hickman, R.K., Golden, A., and Aboleneen, H. Analysis of *Escherichia Coli* Recombinant Protein Expression by MALDI-TOF Mass Spectrometry of Bacterial Colonies. Biotechniques 28: 890-895 (2000).

BOOK CHAPTERS AND REVIEWS

1. **Winkler, M.A.**, DeWitt, L., and Cheung, W.Y. Calmodulin and calcium channel blockers. Hypertension 9:217-223 (1987).
2. Marshall, G.D., Foglesong, P.D., Price, J.O., **Winkler, M.A.**, and West, W.H. Increased antigen-specific lytic activity of interleukin-2 activated cells by heteroconjugate antibodies. Immunology and Allergy Clinics of North America 8:126-128 (1988).
3. Pan, A.A. and **Winkler, M.A.** The Threat of Chagas' Disease in Transfusion Medicine. Laboratory Medicine, 28: 269-274 (1997).

ABSTRACTS

Fifteen abstracts have been published of presentations at major national and international scientific meetings. Detailed list available on request.

PATENTS

1. **Winkler, M.A** and Pan, A.A. Assay for *Trypanosoma cruzi* antibodies which specifically bind three different antigens. U.S. Patent No. 5,550,027. Issued 8/27/96.

2. **Winkler, M.A.** and Pan, A.A. Process for Purifying the Gp 60/50 Antigen of *T. cruzi.* U.S. Patent No. 5,583,204 issued 12/10/96.

3. **Winkler, M.A.** and Pan, A.A. Process for linking an antigenic glycolipid of *T. cruzi* to a protein carrier. U.S. Patent No. 5,623,058 issued 4/22/97.

4. **Winkler, M.A.** and Pan, A.A. Assay for Chagas' Disease and reagents for its use. U.S. Patent No. 5,645,838 issued 7/08/97.

AWARDS AND HONORS

Who's Who, American Men and Women of Science
Abbott Laboratories Chairman Award, 2000.

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REFERENCES

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